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Re-inventing the Neural Crest: Direct Reprogramming makes iNCCs

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Abstract

Aberrant neural crest (NC) development is at the origin of many congenital diseases. Given the limitations in human NC cell isolation and expansion, the development of new strategies for NC generation is crucial. In this issue, Kim et al. report the direct reprogramming of postnatal fibroblasts into multipotent NC cells.

Main text

Neural crest (NC) cells occupy a unique position among stem cell populations found in the embryo. First, the NC innovation was a crucial event in vertebrate evolution by making possible the development of paired sense organs and, with the development of NC-derived jaws, the transition to a more predatory life style. Second, the NC has one of the broadest differentiation potentials *in vivo*, surpassed only by the inner cell mass of the blastocyst (Bronner and LeDouarin, 2012). As a consequence, anomalous NC development is at the onset of many congenital diseases including craniofacial, cardiovascular and bowel syndromes (Takahashi et al., 2013). Aberrant NC biology has also been associated with cancer originating from NC-derived tissues, such as melanoma, the most aggressive skin cancer (Shakhova et al., 2012). Understanding how NC cells develop and generate their distinct progeny is thus highly relevant. In this issue, Kim and colleagues report the direct reprogramming of postnatal fibroblasts into induced neural crest cells (iNCCs). This approach constitutes an excellent platform to investigate NC biology in development and disease (Kim et al., 2014).

The NC is a transient stem cell population that emerges from the dorsal margin of the neural plate in vertebrate embryos. During neural tube closure, NC cells undergo an epithelial to mesenchymal transition (EMT) and migrate extensively to populate different parts of the embryo. In their target structures, they give rise to a plethora of cell types and tissues, such as the ectomesenchyme of the craniofacial elements, glia and neurons of the peripheral nervous system (PNS), melanocytes in the skin, and smooth muscle cells of the outflow track of the heart.

The existence of cells with NC potential is not restricted to early embryonic development since NC-like cells have been identified in NC-derived tissues during the fetal period and adulthood of various vertebrate species, including human. Moreover, NC-like cells have been isolated from many sources, such as skin, sciatic nerve, gut, and bone marrow. Although they display cell intrinsic differences depending on the source and timing of isolation, post-migratory NC-like cells are capable of differentiating into several neural and non-neural cell types. Therefore, given their broad developmental

potential and their accessibility in adult organs, it has been proposed that NC-like cells might have therapeutic value. However, a major limitation for the use of post-migratory NC-like cells is their restricted availability and expandability (Dupin and Sommer, 2012).

Given the broad range of diseases with NC etiology and the restrictions in the isolation of human NC, the development of new model systems for the generation of this cell population is imperative. Such models should provide good platforms not only to study the mechanisms underlying NC self-renewal and cell fate specification but also for human disease modeling. In the last decade, a major effort has focused on the derivation of NC cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Several studies have reported the differentiation of NC derivatives from hESCs or iPSCs, including melanocytes, nociceptive neurons, and autonomic neurons (Mica et al., 2013; Lee et al., 2009; Chambers et al., 2012). For instance, Lee and co-authors reported the derivation of patient-specific iPSCs and their differentiation into various NC derivatives, including PNS neurons. The patients suffered from familial dysautonomia (FD), a rare PNS neuropathy caused by a mutation in the IKBAKP gene. The derived NC progenitors expressed low levels of the IKBAKP transcript, suggesting that the differentiation protocol applied recapitulated specific mechanisms of the disease.

Kim and colleagues (Kim et al., 2014) now report a major advance in this direction by directly reprogramming human postnatal fibroblasts into iNCCs. In brief, the authors used a Sox10*::*eGFP reporter hESC line to determine which factors are sufficient to induce NC from hESC-derived fibroblasts. The transcription factor Sox10 is broadly expressed in undifferentiated NC cells during development and in adulthood and plays a crucial role in NC cell maintenance, survival, and lineage-specific differentiation (Britsch et al., 2001). Therefore, Kim and colleagues postulated that Sox10 would be required for iNCC induction. By using an inducible Sox10 expression system they observed that Sox10 overexpression in combination with extracellular matrix components and epigenetic modifiers was sufficient to activate GFP expression in Sox10*::*eGFP fibroblasts (Figure 1). They speculated that Wnt signaling might further promote NC formation, since Wnt is one of the main morphogens for NC induction at the neural plate border (Groves and LaBonne, 2014). Indeed, by adding the Wnt agonist Chir 99021 to the induction protocol they were able to increase the number of putative iNCCs (Figure 1). The iNCCs displayed NC-enriched gene signatures comparable to that observed in hESC-derived NC cells and were able to self-renew for several passages, even after withdrawal of exogenous Sox10. Interestingly, by using an Oct4*::*eGFP reporter system

Kim and co-authors demonstrated that their iNCC reprogramming protocol does not involve a pluripotent intermediate stage.

To apply their iNCC induction protocol to postnatal human fibroblasts the authors found that expression of the surface marker CD34 allows segregation of iNCCs from non-reprogrammed cells. *In ovo* and *ex vivo* transplantation assays in chicken embryos revealed that fibroblast-derived iNCCs were able to migrate and integrate into NC-derived tissues, such as the dorsal root and sympathetic ganglia and the enteric nervous system.

Transplantation experiments of this kind leave open the question of whether the transplanted cell population is indeed composed of multipotent cells with stem cell features or rather a mixture of restricted progenitors. Likewise, quail-chick transplantation experiments performed several years ago were fundamental in demonstrating that the NC as a population is highly plastic and able to respond to external cues by adopting different fates (Dupin and Sommer, 2012). However, such transplantation experiments cannot exclude mosaicism or re-activation of a multipotency program upon transplantation. The developmental potential of a cell has, therefore, to be addressed by clonal assays. Of note, the authors performed *in vitro* clonal experiments, in that single GFP-labelled iNCCs were co-cultured with other iNCCs. About 60% of the clones analyzed were multifated, indicating that their protocol enables the generation of multipotent iNCCs. These findings are in agreement with classical clonal assays of avian and mammalian NC cells that have previously provided compelling evidence for multipotency *in vitro* (Dupin and Sommer, 2012).

The NC is a product of its embryonic environment and the *in vitro* and transplantation conditions most likely do not reiterate the milieu of the developing embryo. For instance, although NC multipotency *in vitro* has been comprehensively established, the question of whether NC cells are indeed multifated *in vivo* or rather a mixture of pre-specified progenitors remains highly controversial. This uncertainty also raises the question of whether the iNCCs obtained in this study are actually equivalent to their endogenous *in vivo* counterparts. This point might be relevant if iNCCs shall be used to study mechanisms of fate specification and cellular differentiation or to pursue functional cell replacement strategies in the future.

In any case, the iNCCs generated by Kim and co-authors constitute a very valuable platform for the detection of new mechanisms underlying NC-related diseases. Indeed, in this study the authors not only showed that FD-patient iNCCs displayed low levels of the IKBAKP transcript most likely due to alternative splicing, but also identified novel splicing variants in two core NC transcription factors (Figure 1). It will be interesting to

use iNCCs in large-scale drug screenings to identify new molecules capable of reversing the phenotype of this and other diseases. Thus, iNCCs hold great potential for cell-based therapies. To realize this potential, however, the genomic integration of retrovirally transduced Sox10 must be circumvented, for instance with chemical reprogramming. Additionally, it is crucial that the functionality of newly generated cell types will be validated in *in vivo* disease models.

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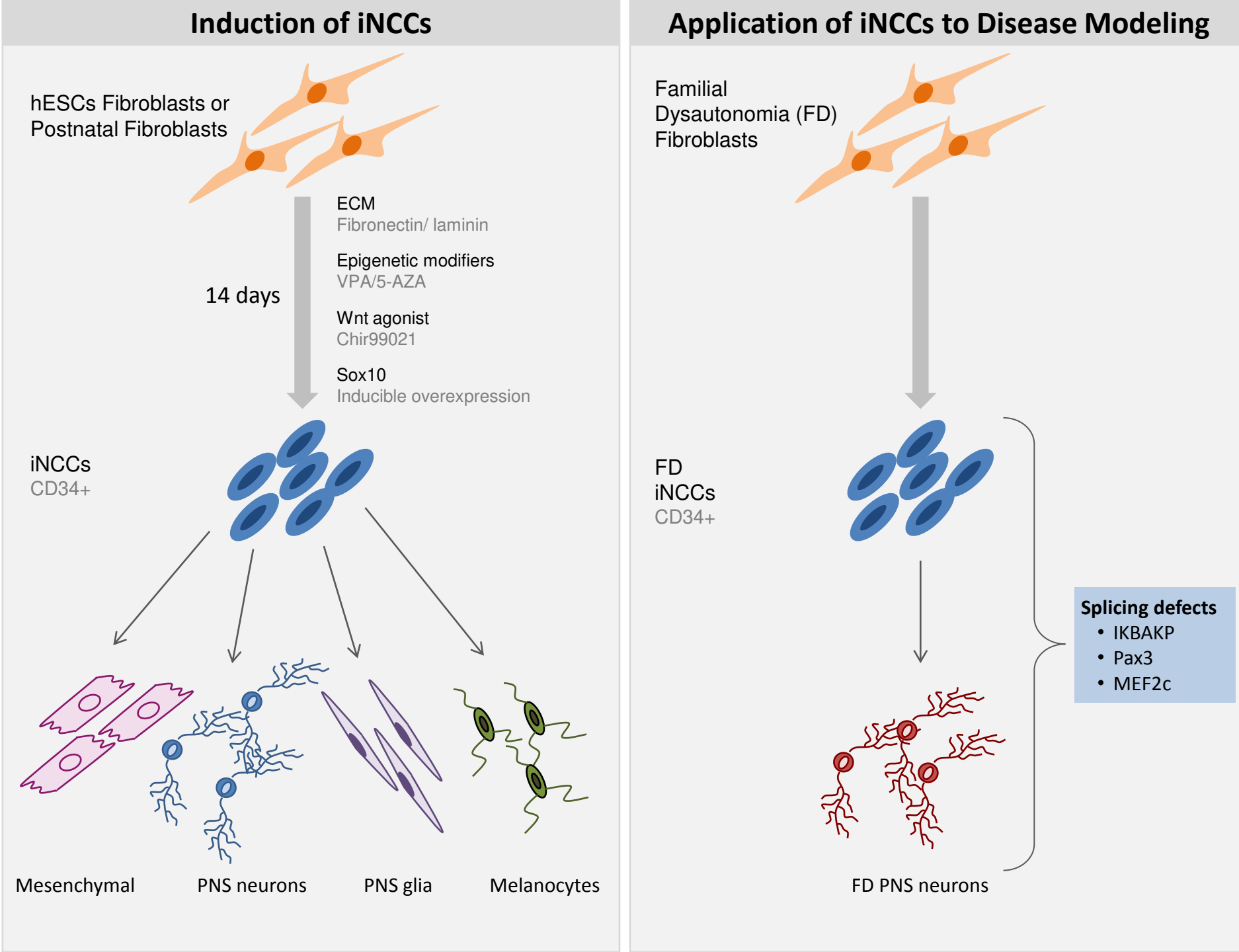
Figure Legends

Figure 1. Direct reprogramming of fibroblasts into iNCCs and their application in disease modeling.

Fibroblasts can be reprogrammed into iNCCs by transient Sox10 overexpression and a combination of extracellular matrix (ECM) components, epigenetic modifiers and the Wnt agonist Chir99021. iNCCs express CD34 and can be differentiated into various NC derivatives (Left side). This approach represents an excellent platform for the modeling of NC-related diseases. Indeed, application of this induction protocol to familial dysautonomia (FD) fibroblasts revealed that iNCCs derived from FD patients displayed aberrant splicing variants of IKBKAP, PAX3 and MEF2c transcripts (Right side).

Abbreviations: hESCs (human embryonic stem cells); iNCCs (induced neural crest cells); 5-AZA (5-aza-2'deoxycytidine); VPA (valproic acid); PNS (peripheral nervous system).


Induction of iNCCs
hESCs Fibroblasts or
Postnatal Fibroblasts
14 days
ECM
Fibronectin/ laminin
Epigenetic modifiers
VPA/5-AZA
Wnt agonist
Chir99021
Sox10
Inducible overexpression
iNCCs
CD34+
Mesenchymal
PNS neurons
PNS glia
Melanocytes
Application of iNCCs to Disease Modeling
Familial
Dysautonomia (FD)
Fibroblasts
FD
iNCCs
CD34+
Splicing defects
• IKBAKP
• Pax3
• MEF2c
FD PNS neurons